IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Holger BOCK and Thomas LINDHORST
Serial No.	:	09/914,052
For	:	**OLIGOMERS SUBSTITUTED BY PHOSPHITE ESTER, PHOSPHONIC ACID OR CARBABORANE FUNCTIONS AND THE CORRESPONDING PNA MONOMERS**
Filed	:	November 20, 2001
Examiner	:	Patrick T. Lewis
Art Unit	:	1623

745 Fifth Avenue, New York, NY 10151

EXPEDITED PROCEDURE
RESPONSE AFTER FINAL ACTION
UNDER 37 C.F.R. §1.116

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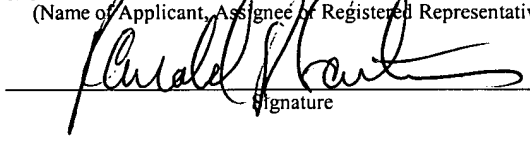
(Name of Applicant, Assignee or Registered Representative)



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DECLARATION UNDER 37 C.F.R. §1.132

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Dear Sir:

This Declaration is filed in support of the concurrently filed Response after Final Action, which is filed in response to the Final Office Action of December 29, 2004. A Notice of Appeal is also being filed concurrently. The following Declaration is made by Thomas Lindhorst, one of the inventors of the present application.

DECLARATION

In the United States Patent Office

In re application of

Holger BOCK, Thomas LINDHORST

Application no. 09/914,052

International filing date: March 3, 200C

Title: Oligomers substituted by phosphite ester, phosphonic acid, or carbaborane functions and the corresponding PNA monomers

1. I, Thomas Lindhorst, of Sieberersträße 3, A-6020 Innsbruck, Austria, am Chief Scientific Officer of Ugichem GmbH, Innsbruck, Austria. I am also a chemist and my field of research and expertise is in the area of organic and medicinal chemistry.

I have carefully studied and fully understood the prior art references

a) Egholm, Michael *et al.* (1992) Peptide Nucleic Acids (PNA). Oligonucleotide Analogues with an Achiral Peptide Backbone, Journal of the American Chemical Society, 114(5), 1895-1897

b) Varadarajan, Aravamuthan and Hawthorne, M. Frederick (1991) Novel Carboranyl Amino Acids and Peptides: Reagents for Antibody Modification and Subsequent Neutron-Capture Studies, Bioconjugate Chemistry, 2(4), 242-253

c) Kane, Robert R. *et al.* (1993) Solution-Phase Segment Synthesis of Boron-Rich Peptides, Journal of Organic Chemistry, 58(5), 991-992

d) US 5,846,741 to Griffiths *et al.*, Boron Neutron Capture Therapy Using Pre-Targeting Methods, December 8, 1998

From my review of the above documents it appears to me that there are a number of misinterpretations in the Office Actions. For the reasons laid out below I do, therefore, not share the Examiner's opinion that the present invention would have been obvious in view of the presented prior art references.

2. The subject-matter of claim 1 of the UgiChem application pertains to a compound which contains at least one peptide nucleic acid (PNA) derivative where the α-carbon of the 2-aminoethyl glycine backbone moiety of said PNA derivative carries the substituents R1 and R2 and at least one of R1 and R2 has one or more radical(s) selected from phosphite ester, phosphonic acid, or carbaborane.

Before I comment specifically on the Office Actions, I will briefly summarize some knowledge about peptide nucleic acids and their potential use in medical applications around the time the UgiChem invention was made. For a review see RAY, A. and NORDEN, B. (2000) Peptide nucleic acid (PNA): Its medical and biotechnical applications and promise for the future, *FASEB J.*, 14, 1041-1060).

2.1 Peptide nucleic acids are DNA mimics with a pseudo peptide backbone. This backbone, which replaces the sugar phosphate backbone of natural nucleic acids, is usually formed by 2-aminoethyl glycine units. To this backbone natural or unnatural nucleobases are connected via a methylenecarbonyl linkage. PNAs are non-ionic, achiral molecules which have been shown to be capable of sequence-specific binding to DNA/RNA and the hybrid complexes exhibit extraordinary thermal stability and unique ionic strength effects. Because of these properties PNAs are considered promising agents for antigene and antisense therapy.

However, the extremely poor cellular uptake of PNAs is the major obstacle against the prospective use of PNAs in such therapeutic applications. This serious problem of cellular uptake of PNAs can mainly be attributed to their poor water solubility. For instance, Wittung, P. *et al.*, FEBS Letters, 1995, 365, 27-29, demonstrated that unmodified PNAs and those modified according to the citation of Egholm *et al.* are not suited to be imported into cells by diffusion.

The problems related to PNA delivery into the cell have been addressed by numerous researchers and several methods to facilitate the uptake of PNAs in eukaryotic cells have been proposed. At the time of the UgiChem invention these included transient permeabilisation with streptolysin O, cell membrane permeabilisation by lysolectin or detergents like Tween, conjugation with peptides, cationic lipid-DNA-PNA complex, incorporation of PNA into delivery vehicles, e.g. liposomes, and modifying the PNA by incorporating C-terminally a positively charged lysine residue (cf. Ray and Norden, l.c.).

2.2 The invention described in the UgiChem US patent application 09/914,052 concerns compounds containing PNA units having phosphite ester, phosphonic acid, or carbaborane functions incorporated, which have been found to possess improved ability to permeate into cells (cf. page 2, 1st full paragraph of the application).

Examples of phosphonic acid radicals, phosphite ester radicals, and carbaborane radicals are given on page 6, paragraphs 4-6 of the application. In this connection it has to be remarked that applicant claims phosphonylated PNAs and not phosphorylated PNAs as alleged on page 7 of the Office Action dated February 25, 2003. In a phosphorylated compound the phosphor is connected to a carbon of the respective compound via an oxygen, while in a phosphonylated compound a direct bond between phosphor and the respective carbon is formed.

As already mentioned above, water solubility is of key importance for cellular uptake of PNAs. This is because cells consist to a large part of water and, at the same time, are mainly surrounded by water.

From Attachment A it can be taken that a PNA-derivative according to the present invention shows a water solubility of at least 659 mg/ml. In comparison, the Egholm H-T10-Lys-NH_2 PNA has a solubility of about 5 mg/ml in water. Hence, the UgiChem PNA shows a significantly improved water solubilty. Specifically, the UgiChem PNA is 130 times more soluble in water than the Egholm H-T10-Lys-NH_2 PNA.

Furthermore, Attachment B shows that biotinylated UgiChem PNAs are also readily imported into cells. Again, from

previous reports of standard PNA chemistries (see e.g. RAY and NORDEN for a review, l.c.) it is known that there is almost no cellular import. Thus, PNAs according to the UgiChem application do not only possess a significantly increased water solubility but do also achieve a far greater level of cellular import than is seen with the standard PNA chemistries according to Egholm.

The uptake of the UgiChem PNAs is, moreover, not limited to a particular cell type provided that it is an eukaryotic cell. Hence, UgiChem PNAs are excellent agents for use in antigene and antisense therapy, since they are able to penetrate into the nucleus where they can recognize and hybridize to complementary DNA/RNA sequences, e.g. tumour-specific DNA/mRNA sequences, and thereby inhibit both transcription and translation of particular genes, e.g. cancer genes. As UgiChem PNAs can thus accumulate specifically in targeted cells, e.g. cancerous cells, a high concentration of boron becomes specifically localized to said targeted cells, e.g. at tumour sites, if the PNAs are boronated, thereby also allowing to effect treatment of the tumour cells by boron neutron capture therapy (BNCT).

3. The prior art reference Egholm *et al.*, J. Am. Chem. Soc., 1992, 114, 1895-1897 deals with peptide nucleic acids as oligonucleotide analogues with an achiral peptide backbone. In particular, this reference discloses PNAs having a positively charged lysine residue incorporated at the carboxyl terminal. Egholm *et al.* were aware of the problems that PNA molecules have poor water solubility and a tendency to aggregate and, therefore, chose to include a lysine at the C-terminal of the PNA (Egholm *et al.*, page 1896, left column, last paragraph).

As correctly elaborated by the Examiner, Egholm *et al.* does not teach that the introduction of phosphonic acid radicals, phosphite ester radicals, or carbaborane radicals into the 2-aminoethyl glycine backbone unit of PNAs does significantly improve water solubility of PNAs. Again, a 15mer-UgiChem PNA without a C-terminal lysine shows a solubility of at least 659 mg/ml in water (cf. Attachment A). Clearly, this is a remarkable improvement over the H-T10-Lys-NH_2 PNA of Egholm *et al.* which has a fairly poor solubility of 5 mg/ml in water. Thus, for a person skilled in the art the teaching of the UgiChem application is entirely surprising, since the significantly increased water solubility could not be expected. The same is true for the substantially improved cellular uptake. Therefore, the UgiChem application warrants an inventive step over Egholm *et al.*

4. The prior art reference of Varadarajan and Hawthorne, Bioconjugate Chem., 1991, 2, 242-253, reports the synthesis of carboranyl amino acids and peptides. In particular, the chemical synthesis of an α-amino acid derivative incorporating the 1,2-dicarba-*closo*-dodecarborane, peptide derivatives including this modified amino acid and the conjugation of the peptide derivates to an antibody are described.

At page 247, right column, second paragraph, it is reported that the prepared amino acid containing the carborane cage is exceptionally hydrophobic and thus poorly water soluble. Only by converting the *closo*-carboranyl amino acid to the anionic nido derivative water soluble products could be obtained (cf. page 247, left/right column, bridging

paragraph, page 248, right column, last paragraph). Thus, the observation of UgiChem that the introduction of an uncharged carbaborane radical improves the water solubility of PNA stands in sharp contrast to the teaching of this reference. Consequently, it is unclear how this reference could contribute to the UgiChem invention.

Besides, it should be noted that Varadarajan and Hawthorne teaches that boronated antibody conjugates suffer from either significantly reduced immunoreactivity or low tumor uptake. To overcome this problem Varadarjan suggests to boronate small peptides which are subsequently attached to antibodies instead of boronating the antibodies themselves directly. Notably, these small boronated peptides are not biological active themselves. Rather, said peptides are simply used as antibody-tags, and, as commonly known, antibodies bind to antigens which are localized on the cell surface. This, clearly, constitutes an entirely different solution than the PNAs of the instant invention. Ugichem PNAs penetrate the cell membrane and are biological active themselves by binding to RNA instead of antibody-tags which is clearly a different activity than that teached by Varadarajan and Hawthorne. Accordingly, the present invention is based on an inventive step over Varadarajan and Hawthorne.

Moreover, the article of Varadarajan and Hawthorne does not contain any description of PNA nor does it mention or suggest the derivatisation of PNA with carbaborane radicals, phosphonic acid radicals or phosphite ester radicals in order to improve the water solubility of PNA. Therefore, the superior properties of the UgiChem PNAs, i.e. significantly improved water solubility and cellular uptake, could neither

be predicted nor were they obvious from the teaching of Varadarajan and Hawthorne. From this it directly follows that the present invention does involve an inventive step in view of the citation of Varadarajan and Hawthorne.

5. The reference of Kane *et al.*, J. Org. Chem., 1993, 58, 991-992, also refers to the synthesis of carborane-containing peptides. In particular, Kane *et al.* describes a peptide in which a carborane containing amino acid and glycine are alternately connected to each other.

At page 992, right column, second paragraph, it is noted that the current work is concerned with the incorporation of hydrophilic amino acids (glutamic acid or phosphorylated serine) in order to increase the hydrophylicity of the derived peptides. However, no results are presented which would substantiate this theoretical consideration of increasing the hydrophilicity by incorporating hydrophilic amino acids. Thus, from this reference one does not gain any information going beyond the disclosure of Varadarajan and Hawthorne which is in contradiction to the UgiChem invention as outlined above under item 4.

In addition, the peptides described by Kane *et al.* are entirely different from the PNAs of the UgiChem application. First, the UgiChem application is concerned with PNA derivatives and thus with a different class of compounds. Second, it is important to note that a PNA can only function as a nucleic acid analogue if the spacing between the nucleobases resembles the distance between nucleobases of a natural nucleic acid. Such resemblance requires that the PNA backbone has to be homomorphous with the sugar phosphate backbone of a natural nucleic acid. By convention, this

requirement is fulfilled if the PNA backbone is formed by repeated 2-aminoethly glycine units.

Now, incorporating hydrophilic amino acids into the backbone structure of a PNA by alternately connecting said amino acids to the 2-aminoethyl glycine moiety of a PNA-unit as suggested by Kane *et al.* would no longer be homomorphous with the sugar phosphate backbone of a natural nucleic acid. Consequently, such a mixed peptide formed from hydrophilic amino acids and PNA-units would, due to the altered spacing of the nucleobases, not be able to recognize and hybridize to complementary DNA/RNA sequences. Hence, a modification of the UgiChem PNAs according to the theoretical considerations of Kane *et al.* in order to increase the hydrophilicity would be deleterious to their function as DNA/RNA analogues.

For the reasons outlined above, a person skilled in the art would, therefore, not turn to this document, which suggests a solution detrimental to the function of PNAs, when faced with the object to improve the water solubility and cellular uptake of PNAs. Additionally, Kane *et al.* does not contain any teachings as to how PNAs could be modified or why. The present invention must therefore warrant an inventive step over Kane *et al.*

6. US 5,846,741 is directed to a method for targeting boron atoms to tumour cells for effecting boron neutron capture therapy (BNCT). The method comprises to pre-target tumour cells with an antibody conjugate, followed by targeting the antibody with a boron-containing compound which specifically recognizes and binds to the conjugate of the antibody. Subsequently the tumour cells can be destroyed by

irradiating the boron atoms of the boron-containing compound, thereby effecting BNCT.

First, it should be noted that there is a substantial difference between the UgiChem PNAs and the method disclosed by Griffiths *et al.* in that both the antibody and the boron-containing compound are localized on the outside of the cell membrane of the respective tumour cells while the UgiChem PNAs penetrate into the cell nucleus. Even though Griffiths *et al.* teaches to use complementary polynucleotid fragments including DNA, RNA and synthetic analogues of polynucleotids such as PNAs, the recognition and hybridization to the complementary antibody-conjugate occurs on the outside of the cell membrane of the respective tumour cell. This purpose, however, is exactly the opposite of the aim followed in the UgiChem application, since the object of the UgiChem application is to provide PNAs with improved ability to permeate into cells.

Griffiths *et al.* does, moreover, not mention or suggest any modification of PNAs in order to improve their water solubility, let alone disclosing the particular derivatization of the PNAs according to the UgiChem application.

Therefore, the properties of the UgiChem PNAs could neither be predicted nor were they obvious from the teaching of Griffiths *et al.* From this it directly follows that the UgiChem application does involve an inventive step in view of Griffiths *et al.*

7. Taking the foregoing considerations into account, a person skilled in the art would have had no motivation at all to

combine the teachings of Egholm *et al.*, referring to classical PNAs, with Varadarajan and Hawthorne and Kane *et al.* referring to the synthesis of anionic nido-carborane containing peptides when faced with the object to provide PNAs with increased cell-permeating ability. In fact, the teachings of Varadarajan and Hawthorne and Kane *et al.*. would rather point away from the solution of the UgiChem application.

There is also no motivation for one of ordinary skill in the art to combine the teaching of Varadarajan and Hawthorne, which stands in sharp contrast to the UgiChem application (see item 4), and Griffiths *et al.* which contains no hint as to where and how PNAs should be modified in order to improve their cellular uptake. Clearly, a combination of these teachings would not lead to the claimed invention.

In summary, the improved properties of the UgiChem PNAs could neither be predicted nor were they prima facie obvious from the individual teachings or, as argued by the Examiner, from a combination of the teachings of the prior art references cited in the respective Office Actions. Thus, it is believed that the UgiChem application is based on an inventive step with respect to the prior art.

8. I further declare, that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the US Code, and that such wilful false

statements may jeopardize the validity of the application or any patent issuing thereon.

DATE AND SIGNATURE

June, 28th, 2005 [signature]

Enclosures
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